

## ULTRASTRUCTURE OF LYMPHOCYSTIS IN THE HEART OF THE SILVER PERCH, *BAIRDIELLA CHRYSURA* (LACÉPÈDE), INCLUDING OBSERVATIONS ON NORMAL HEART STRUCTURE

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**ABSTRACT** The fine structure of normal heart muscle from the silver perch, *Bairdiella chrysura* (Lacépède), is similar to that previously reported for marine and freshwater teleosts.

Cardiac lymphocystis is a viral disease manifested by single, giant-cell lesions variously located in the epicardium, trabecular spaces, and subendocardium—in direct apposition to myocardial cells. Occasionally, the hyaline capsule of lymphocystis cells partially surround myocardial cells but cause no pathological changes or inflammatory reaction.

The lymphocystis cells contain typical cellular organelles, including the viroplasmic net unique to these cells. Annulate lamellae, often continuous with the rough endoplasmic reticulum, are present, usually along the periphery of the cell. Some elements of the rough endoplasmic reticulum are dilated and contain a finely granular material, but others contain cross-banded fibrils, each having a periodicity of 30 nm. Similar fibrils are present in the perinuclear cisternae.

### INTRODUCTION

Lymphocystis is a non-lethal, giant-cell, viral disease of marine and freshwater teleost fishes. The self-limiting disease is mainly manifested by temporary external tumorous cutaneous lesions. However, lesions have been reported in the eye (Huizinga and Cosgrove 1973, Smith 1973, Lawler et al. 1974, Dukes and Lawler 1975); gastro-intestinal tract, mesenteries and peritoneum (Woodcock 1904, Awerinzew 1909, Bangham and Hunter 1939, Nigrelli and Smith 1939, Smith 1973, Lawler et al. 1974, Russell 1974); ovaries (Woodcock 1904, Awerinzew 1909, Nigrelli and Smith 1939); spleen (Nigrelli and Smith 1939, Huizinga and Cosgrove 1973, Lawler et al. 1974, Russell 1974); liver, gall bladder, kidneys and testes (Lawler et al. 1974, Russell 1974); muscle (Russell 1974); and in the heart (Bangham and Hunter 1939, Lawler et al. 1974).

Lawler et al. (1974) previously reported on the incidence of external and internal lymphocystis infections in silver perch collected from Mississippi Sound. The morphology of cardiac lymphocystis lesions has been given little attention; thus, we have expanded our previous study to include microscopic examination of these lesions in silver perch. Our observations on the ultrastructure of both normal heart and cardiac lymphocystis form the basis of this report.

### MATERIALS AND METHODS

Sixty-two silver perch, *Bairdiella chrysura* (Lacépède), out of 923 collected in 1972 and 1973 from the estuarine waters of Mississippi Sound (30°23'29"N, 88°47'50"W)

exhibited extensive external and/or internal lymphocystis, including five with cardiac lesions (Table 1). Another specimen (M-644) spontaneously developed lymphocystis while retained in an aquarium from May 25 to July 9, 1973, in salinities ranging from 2.8 to 5.6 ppt. It also had extensive external and internal lesions, including cardiac lesions.

Of the six fish, four had an isopod, *Lironeca ovalis* (Say), attached to their gills and another showed evidence of previous infestation. Host data for all silver perch used in this study are summarized in Table 1.

Normal and lymphocystis-infected hearts were prepared for gross and electron microscopic observations in the manner previously described (Lawler et al. 1974). In addition, cardiac tissue was embedded in paraffin and sectioned for staining with hematoxylin and eosin, periodic acid Schiff's (PAS) reaction, Alcian blue, and Masson's method for connective tissue.

The hearts from silver perch M-532 and M-644 were initially fixed in 10% buffered formalin. Tissue was excised and prepared for electron microscopy as if fresh.

Selected heart tissue was flooded with cold 3% glutaraldehyde, minced with a razor blade, and then placed in a larger volume of cold glutaraldehyde for 24 hours. The tissue was then washed in two changes of 0.1M phosphate buffer for 2 hours, post-fixed in cold 1% osmium tetroxide for 2 hours, and dehydrated in a graded series of ethanol over a 1-hour period.

The tissue was embedded in a Maraglas-Cardolite mixture according to the procedures of Freeman and Spurlock (1962). Ultrathin sections were cut with an LKB Ultratome and doubly stained with uranyl acetate and lead citrate. Sections were examined and photographed using a Siemens 1A Elmiskop electron microscope.

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TABLE I.  
Host data for silver perch exhibiting cardiac lymphocystis

Features Observed	Host Number					
	M-530	M-532	M-535	M-628	M-632	M-644
TL <sup>1</sup> Host (mm)	100	104	97	122	138	44
Date Collected	10/6/72	10/11/72	10/14/72	5/29/73	5/30/73	5/25/73 (7/9/73) <sup>2</sup>
Type of Lymphocystis Infection	Natural	Natural	Natural	Natural	Natural	Spontaneous <sup>3</sup>
Isopod Present	L Gills	R Gills	R Gills (previously)	R Gills	L Gills	None
External Lymphocystis						
Head	-	+	+	+	-	+
Nostril	L, R	L, R	L, R	-	-	L, R
Eye (surface)	-	-	R	L	-	R
Jaw	-	-	Lo	-	-	Lo
Mouth	-	-	-	Ro	-	Ro
Operculum	L	L, R	R	R	-	L, R
Gill	L, R	L, R	L, R	L, R	-	L, R
Pseudobranch	L, R	L, R	L, R	-	-	L, R
Isthmus	+	+	-	+	-	+
Body surface	-	R	L, R	L, R	-	L, R
Dorsal fin	B	B	B	B	-	B
Pectoral fin	L, R	-	L, R	L, R	-	L, R
Pelvic fin	L, R	L	L, R	L, R	-	L, R
Anal fin	+	+	+	+	-	+
Caudal fin	-	+	+	-	-	+
Internal Lymphocystis						
Heart	+	+	+	+	+	+
Behind eye	L, R	-	L, R	L, R	-	-
Kidney	L, R	-	L, R	-	-	-
Mesenteries	+	+	+	-	-	+
Spleen	+	+	+	+	-	+
Liver	+	-	+	-	-	+
Ovary	+	-	-	-	?	-

<sup>1</sup>TL = total length

<sup>2</sup>Specimen held in aquarium from date collected and removed on this date

<sup>3</sup>Infection appeared in aquarium

Lo = Lower

R = Right

Ro = Roof

B = Both

L = Left

## RESULTS

### Normal Heart

The walls of the atrium and ventricle generally consist of the epicardium, the myocardium and the endocardium. The myocardium is fairly thick in the ventricle but thin in the atrium, often no thicker than the diameter of a single muscle cell. In some areas, especially in the atrium, there are gaps where the myocardium is absent; thus at those sites the wall consists of epicardium, collagen bundles, and endocardium. The surfaces of the epicardial, myocardial, and endocardial cells are strongly PAS positive and alcianophilic at pH 2.5 and 1.0

**Epicardium.** The epicardium consists of a single uninterrupted layer of epithelial cells underlain by bundles of collagen fibers (Figure 1). The epicardial cells are flattened and each contains a single oval nucleus. The cells form

finger-like cytoplasmic processes which interdigitate with or overlap similar processes of adjacent cells with which they are linked by desmosomes.

**Myocardium.** The atrial myocardium is thin and the branching muscular trabeculae are loosely arranged, whereas the ventricular myocardium is thicker and the trabeculae are more compact. Myocardial cells throughout the two chambers are structurally similar and contain all the organelles usually present in cardiac muscle cells (Figures 2, 3). Each cell contains a central nucleus, and the myofibrils exhibit A, I, and Z bands. The myofibrils tend to be located at the cell periphery and exhibit the pattern of six thin filaments to one thick filament that is typical for vertebrate myocardial cells. The myocardial cells in the atrium, as well as some in the ventricle, contain numerous specific granules.

A transverse tubular system is not present in the myocardial cells but a sarcoplasmic reticulum (SR) is sparsely

scattered throughout, particularly between myofibrils and often in close association with the cell membrane where it forms subsarcolemmal cisternae.

The mitochondria are predominantly located in the cell core but are also scattered between myofibrils. They are often pleomorphic and contain flattened cristae.

The myocardial cells are joined end-to-end by intercalated discs, the gaps of which occasionally contain membranous profiles and osmiophilic granules resembling glycogen (Figure 3). These components are perhaps artifacts of tissue preparation. An occasional macula adherens is encountered in the disc.

**Endocardium.** The endocardium lines both heart chambers and consists of an uninterrupted layer of endocardial cells underlain by large bundles of collagen fibers (Figures 1, 2). The endocardial cells are larger in the atrium where they protrude into the heart chamber. In both chambers these cells are packed with an elaborate smooth endoplasmic reticulum (SER) and contain numerous residual bodies, which are evidently lipofuscin (Howse and Welford 1972).

#### Cardiac Pathology

Lesions consisting of aggregations of lymphocystis cells, common in external manifestations, are not present in the heart. The distribution of the lymphocystis cells in each heart is given in Table 2. Many of the cells are each surrounded by an outer cellular envelope consisting of several layers of epithelioid cells (Figures 4–6, 8, 9).

**Cellular Envelope.** The cellular envelope is composed of uninucleate cells connected by desmosomes. The cells contain the usual organelles; however, adjacent cells frequently vary in the amount of smooth and rough endoplasmic reticulum present. Some cells possess a prominent SER, whereas others exhibit an elaborate rough endoplasmic reticulum (RER) (Figure 9). Some cells differ also in having pigment granules, lipofuscin, and fibrillar material in the perinuclear region. These cells are evidently fibroblasts.

**Lymphocystis Lesions.** Individual lymphocystis cells are variously encountered in the epicardium (Figure 4), the trabecular spaces (Figure 5), and subendocardium—in direct apposition to the myocardial cells (Figure 6). They contain numerous virions scattered throughout the cytoplasm among

the usual cellular organelles, including viroplasmic nets unique to these cells (Figures 6, 7). The fine structure of the viroplasm is similar to that of nuclear chromatin except that a delimiting membrane is not present.

Mitochondria are numerous and varied in size and shape. Their cristae are sparse. Smooth endoplasmic reticulum is abundant in some cells, especially in the cortical cytoplasm, but is poorly defined in others.

The RER is elaborate, especially along the cell periphery (Figure 8). Frequently, the RER is markedly dilated and contains a fine granular material of electron density similar to that of the hyaline capsule, but other regions of the RER contain numerous cross-banded fibrils (Figures 8, 12, 13). These bands exhibit a periodicity of about 30 nm. Occasionally, these fibrils extend into the granular material contained in the dilated RER (Figure 13). Cross-banded fibrils are also occasionally present in the perinuclear cisterna (Figure 12).

Annulate lamellae are present in the peripheral cytoplasm and consist of parallel RER cisternae exhibiting pore-annuli in register with each other (Figures 14–16). The inter-cisternal regions are from 80 to 110 nm wide and contain osmiophilic granular material. These lamellae are occasionally continuous with membranes of the RER (Figures 14, 15).

The lymphocystis cell surface is marked by numerous deep invaginations filled with hyaline (Figures 8, 10). The hyaline capsule gives the usual reactions of mucopolysaccharides to the histochemical stains employed in this study. They are PAS and Alcian blue positive, and are light blue following Masson's procedure for connective tissues. The capsules consist of an amorphous matrix in which numerous fine fibrils are embedded (Figures 8–10).

Occasionally, the hyaline capsule at least partially engulfs a myocardial cell (Figure 10), but this close relationship causes no histopathological changes in the muscle cell. No unusual structural changes are evident in the myocardial cells nor are inflammatory cells present in the immediate intercellular spaces. These spaces occasionally contain a finely granular material.

Virions are rarely present in the interstices (Figure 11). The radial-fibrillar region that typically surrounds intra-

TABLE 2.  
Number and location of cardiac lymphocystis cells (LC) in four silver perch. The numbers enclosed in parentheses represent the numbers encased in a cellular envelope.

Specimen	Total LC	Atrial Endocardium	Atrial Epicardium	Ventricular Endocardium	Ventricular Epicardium
M-632	8 (8)	0	4 (4)	4 (4)	0
M-532	30 (11)	5 (1)	1 (1)	15	9 (9)
M-530	3 (2)	1 (1)	1 (1)	1	0
M-535	8 (6)	1	1 (1)	4 (2)	3 (3)

cellular virions is also present but denser about the interstitial virions.

Frequently, hyaline extensions of the capsule weave tortuous courses among the cells of the envelope (Figures 4-6). The fine structure of the extensions is similar to that of the hyaline capsule except that some of the extensions contain collagen fibers. These fibers have a periodicity of about 70 nm and are particularly evident in hyaline extensions when viewed longitudinally.

#### DISCUSSION

The fine structure of the normal silver perch heart is similar to that described for other marine and freshwater fish species. However, the available information is sparse and fragmentary, except for a detailed account of the heart of the freshwater teleost, *Oryzias latipes* (Schlegel), by Lemanski et al. (1975) which presents an excellent discussion of the normal heart structure in a fish.

Although the present study dealt mainly with cardiac lymphocystis, internal lesions were present throughout the several organ systems of the perch. Our findings, together with those previously cited, suggest that internal lymphocystis infections are not as rare as previously believed (Walker 1957). The wide-spread occurrence of lymphocystis in the organ systems of perch supports the suggestion that internal lymphocystis may develop *in situ* rather than represent "displaced elements" (Nigrelli and Ruggieri 1965).

Lymphocystis appears to have a systemic phase as well as a localized external response phase. Over 30 years ago, Weissenberg (1945) suggested that the virus can gain entrance into the host vascular system via the gills. More recently, Smith (1973) and Duker and Lawler (1975) observed lymphocystis in certain ocular tissues which seems to preclude all but a hematogenous mode of infection. The present rare finding of viral particles free in the interstitial space of the myocardium of perch further supports this view.

Of particular interest in this study are the findings of annulate lamellae located in the peripheral cytoplasm, and

the fibril-containing RER and perinuclear cisternae in the lymphocystis cells. Annulate lamellae have been observed in the cytoplasm of a variety of metabolically active cell types. They are present in male and female germ cells of certain invertebrates and vertebrates, embryonic and adult somatic cells, and frequently occur in tumor and cancer cells (see reviews by Kessel 1968, Wischnitzer 1970). Although the function of annulate lamellae remains obscure, the bulk of the evidence suggests that they are involved in protein synthesis (Wischnitzer 1970). The peripheral location of the annulate lamellae and their structural continuity with the RER in lymphocystis cells suggests a role in both the synthesis of the fibrils present in the RER and perinuclear cisternae and the synthesis of the proteinaceous component of the hyaline capsule which is known to be rich in acid mucopolysaccharides (Pritchard and Malsberger 1968, Howse and Christmas 1970).

During the course of accelerated metabolism and rapid hypertrophy of the infected cell, fibrils or their precursor macromolecules are evidently extruded by the cell and become embedded in the hyaline component of the capsule. However, the aggregation of these precursor macromolecules within the cisternae may provide the close association necessary to promote interactions between them that leads to the synthesis of cross-banded fibrils. Since no cross-banded fibrils having a periodicity of 30 nm were encountered in the capsule their presence in the RER and perinuclear cisternae may reflect the loss of the capability of these organelles to expel their products. This may result from an accelerated protein synthesis that overloads the extrusion capacity of these organelles, or the lymphocystis cell may have reached the point of incompatibility between further growth and the available supply of metabolic nutrients; thus, the result is stasis, cell degeneration, and necrosis.

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#### LITERATURE CITED

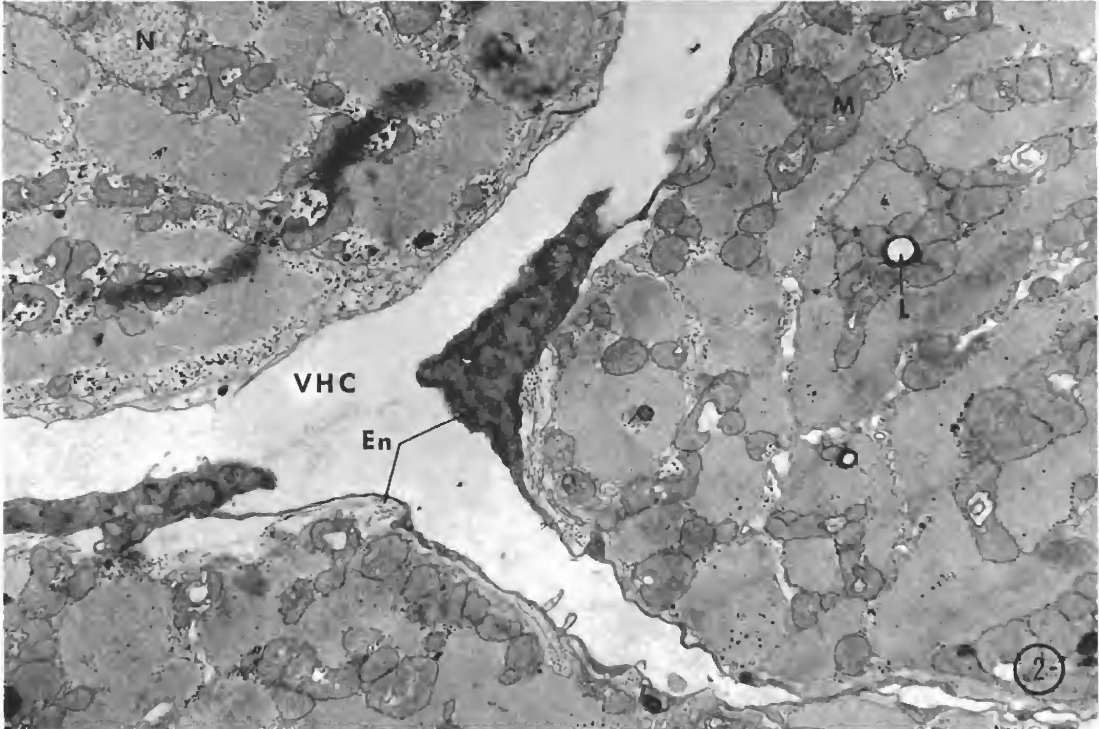
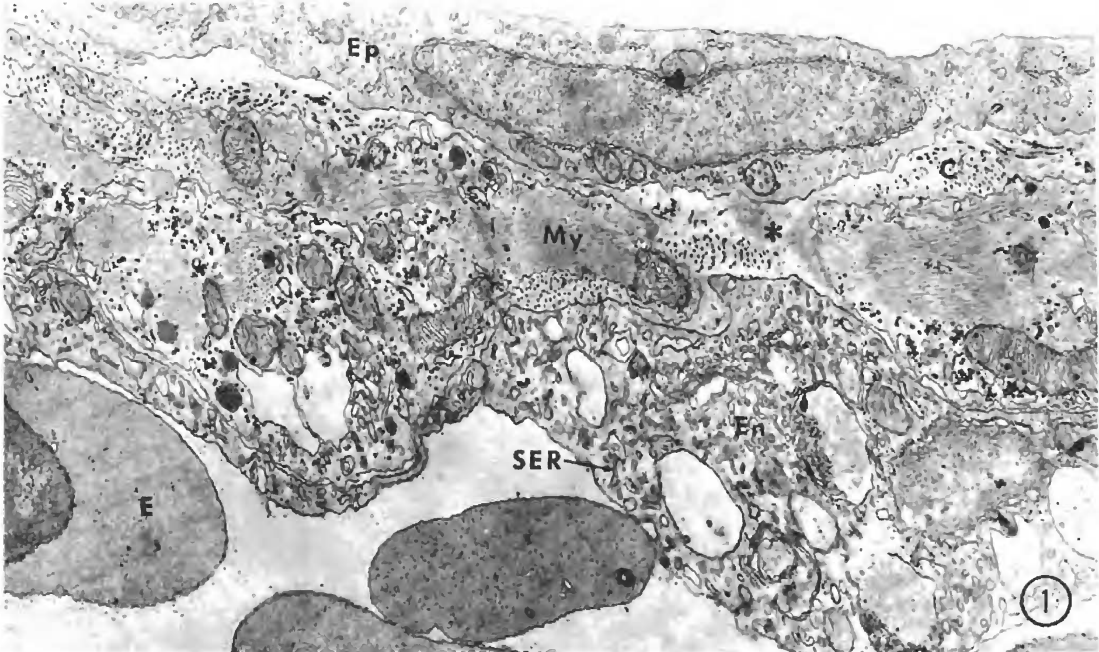
- Awerinzew, S. 1909. Zur Kenntniss von *Lymphocystis johnstonei*. *Zool. Anz.* 31:881-884.
- Bangham, R. V. & G. W. Hunter III. 1939. Studies on fish parasites of Lake Erie. Distribution Studies. *Zoologica* 24:383-448.
- Dukes, T. W. & A. R. Lawler. 1975. The ocular lesions of naturally occurring lymphocystis in fish. *Canadian J. Comp. Med.* 39: 406-410.
- Freeman, J. & B. Spurlock. 1962. A new epoxy embedment for electron microscopy. *J. Cell Biol.* 13:437-443.
- Howse, H. D. & J. Y. Christmas. 1970. Lymphocystis tumors: Histochemical identification of hyaline substances. *Trans. Amer. Micros. Soc.* 89:276-282.
- Howse, H. D. & R. F. Welford. 1972. A histochemical and ultrastructural study, especially of the lipofuscin in the endothelium of the toadfish endocardium. *Trans. Amer. Micros. Soc.* 91: 24-35.
- Huizinga, H. W. & G. E. Cosgrove. 1973. Surface and visceral lymphocystis disease in a white crappie, *Pomoxis annularis*. *J. Wildlife Diseases* 9:349-351.
- Kessel, R. G. 1968. Annulate lamellae. *J. Ultrastruct. Res.* Suppl. 10, 82 pp.
- Lawler, A. R., H. D. Howse & D. W. Cook. 1974. Silver perch, *Bairdiella chrysura*: New host for lymphocystis. *Copeia* 1:266-269.
- Lemanski, Larry F., E. Payson Fitts, & Barry S. Marx. 1975. Fine structure of the heart in the Japanese Medaka, *Oryzias latipes*. *J. Ultrastruct. Res.* 53:37-65.
- Nigrelli, R. F. & G. M. Smith. 1939. Studies on lymphocystis disease in the Orange Filefish, *Ceratacanthus schoepfi* (Walbaum), from Sandy Hook Bay, N. J. *Zoologica* 24(9):255-264.

- Nigrelli, R. F. & G. D. Ruggieri. 1965. Studies in virus diseases of fishes. Spontaneous and experimentally induced cellular hypertrophy (Lymphocystis disease) in fishes of the New York Aquarium, with a report of new cases and an annotated bibliography 1874-1965. *Zoologica* 50:83-96.
- Pritchard, H. N. & R. G. Malsberger. 1968. A cytochemical study of lymphocystis tumors in vivo. *J. Exp. Zool.* 169:371-380.
- Russell, P. H. 1974. Lymphocystis in wild plaice *Pleuronectes platessa* (L.) and flounder, *Platichthys flesus* (L.), in British coastal waters: A histopathological and serological study. *J. Fish Biol.* 6:771-778.
- Smith, G. F. 1973. Lymphocystis disease in *Cynoscion nothus*, *Cynoscion regalis* and *Stellifer lanceolatus* from Georgia estuaries. *Proc. 26th Ann. Conf. Southeastern Assoc. of Game & Fish Comm.* Pp. 598-608.
- Walker, R. 1957. Warty Walleyes. *N. Y. State Conservationist* 12: 28-29.
- . 1962. Fine structure of lymphocystis virus of fish. *Virology* 18:503-505.
- & R. Weissenberg. 1965. Conformity of light and electron microscopic studies on virus particle distribution in lymphocystis tumor cells of fish. *N. Y. Acad. Sci.* 126:375-385.
- Weissenberg, R. 1945. Studies on virus diseases of fish. IV. Lymphocystis disease in Centrarchidae. *Zoologica* 39:169-187.
- Wischnitzer, S. 1970. The annulate lamellae, pp. 65-100. In: G. H. Bourne and J. F. Danielli (ed.), Vol. 27, *International Rev. of Cytol.* Academic Press, New York.
- Woodcock, H. M. 1904. Note on a remarkable parasite of plaice and flounders. *Trans. Liverpool Biol. Soc.* 18:143-152.

## PLATE I

## EXPLANATION OF FIGURES

1. Electron micrograph of the normal atrial wall showing the epicardium (Ep), myocardium (My), and endocardium (En). Note gap (\*) in the myocardium. C, collagen; E, erythrocyte; SER, smooth endoplasmic reticulum. X 26,700.
2. Electron micrograph of normal ventricular trabeculae showing the myofibrils in transverse and oblique views. En, endocardium; VHC, ventricular heart chamber; L, lipid droplet; M, mitochondria; N, nucleus. X 9,500

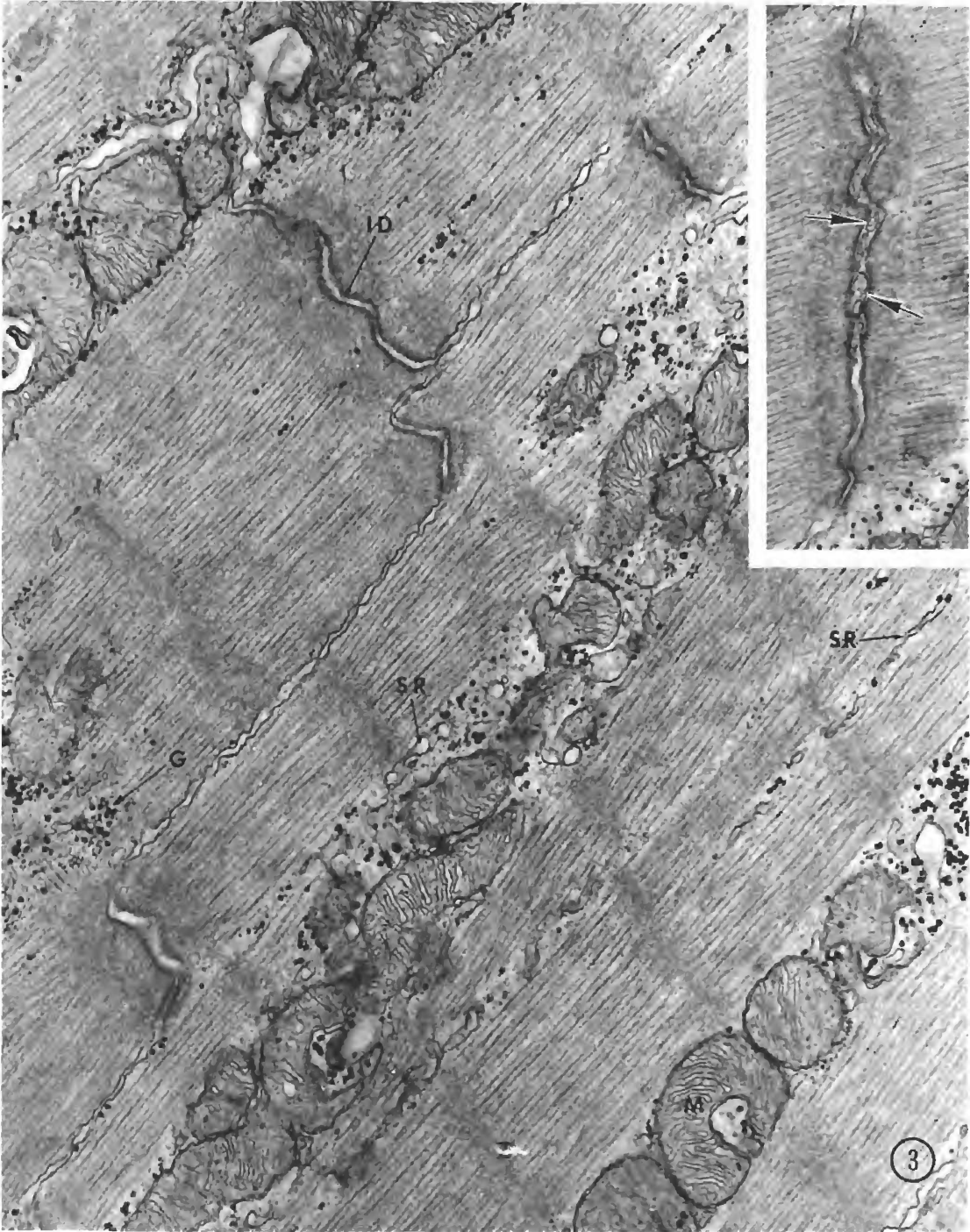


## PLATE 2

## EXPLANATION OF FIGURE

3. Normal myocardial cells sectioned longitudinally in the region of an intercalated disc (ID). G, glycogen; M, mitochondria; SR, sarcoplasmic reticulum; Z, Z bands. X 35,900. Inset: Section through an intercalated disc showing membranous profiles (arrows) in the gap. X 31,900.

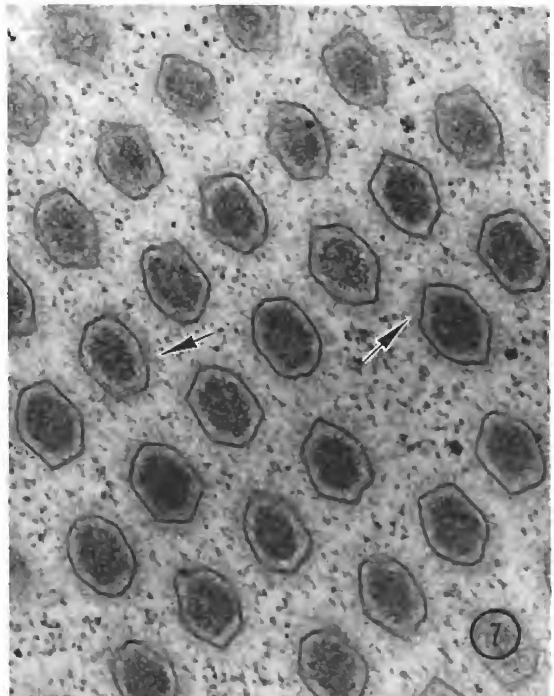
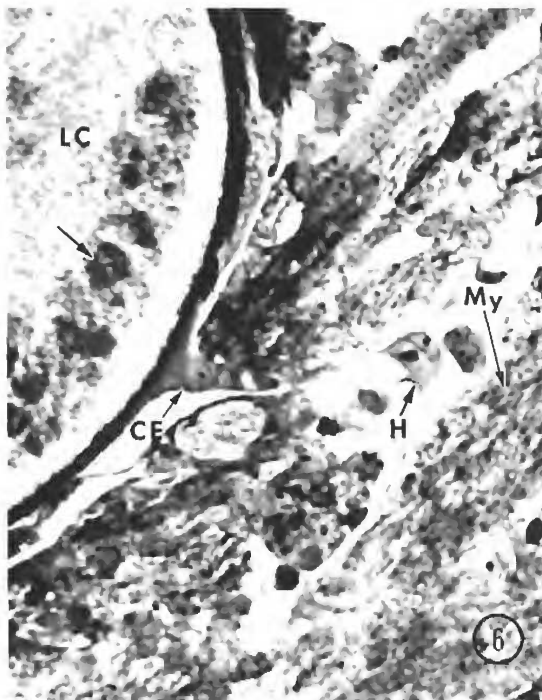
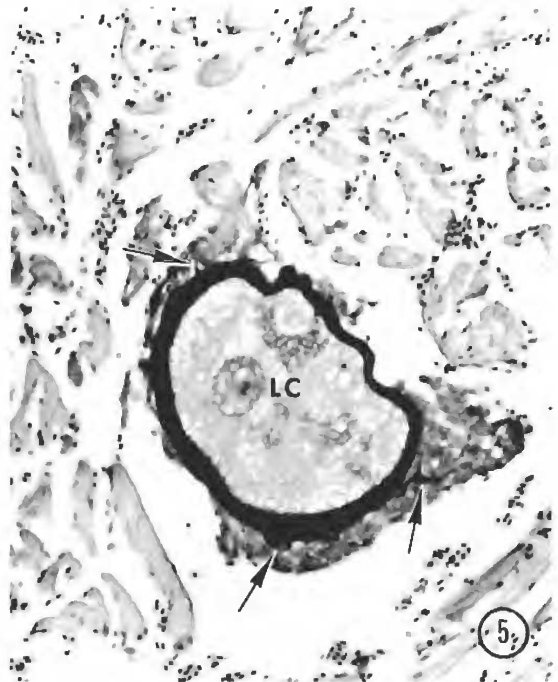
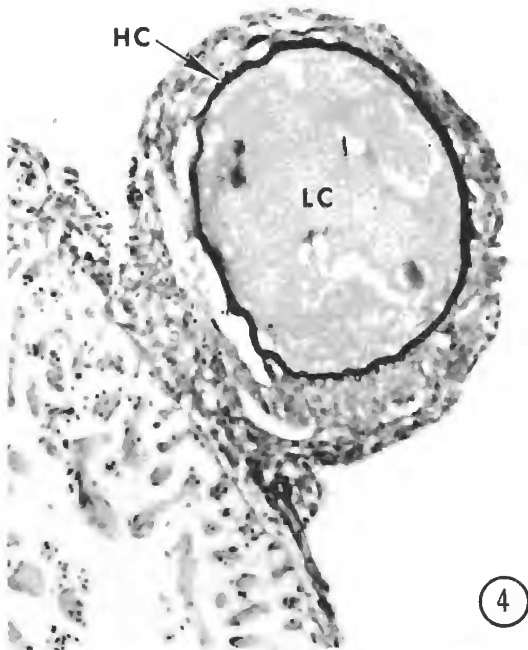




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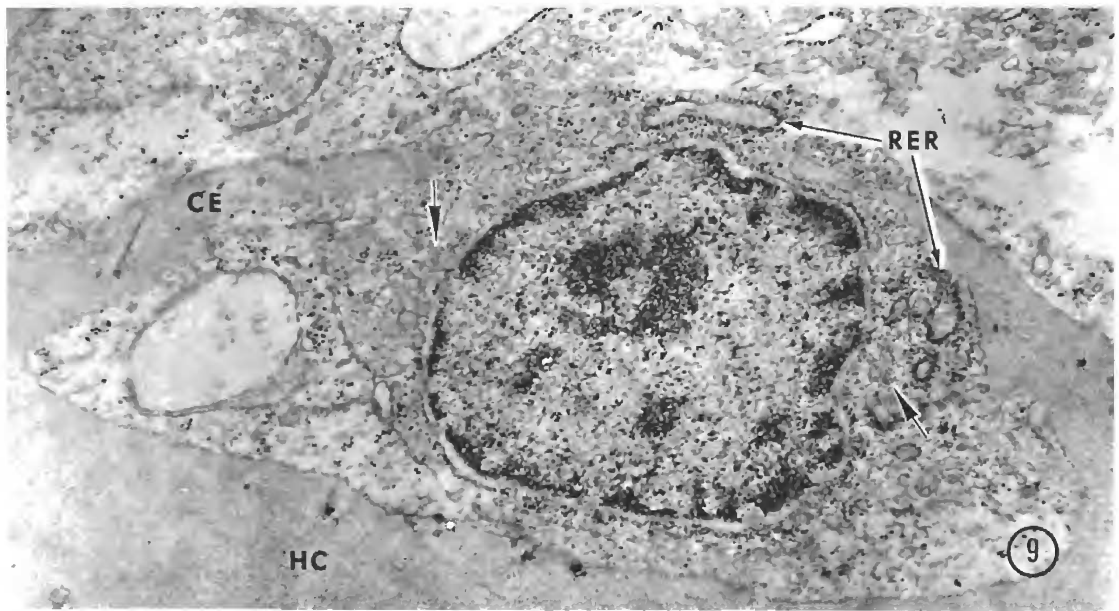
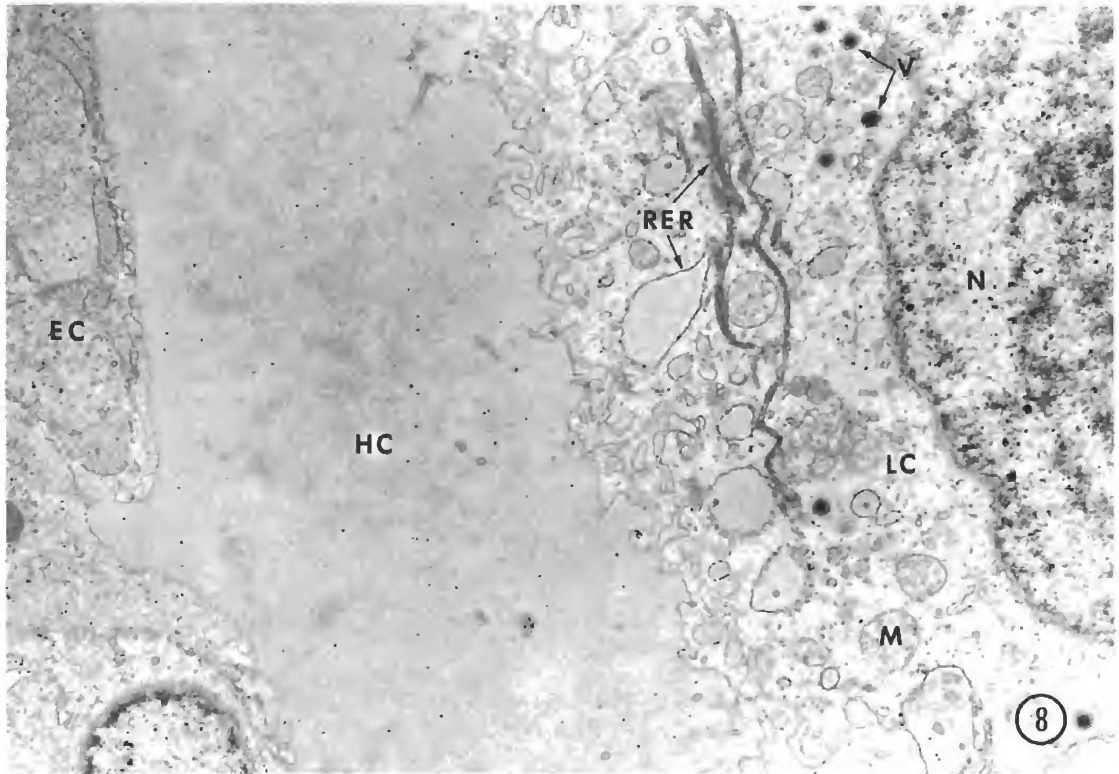
## EXPLANATION OF FIGURES

4. Histological section showing a lymphocystis cell (LC) encapsulated in a cellular envelope attached to the atrial epicardium. Note darkly stained hyaline capsule (HC). Stained with PAS. X 330.
5. Histological section through a lymphocystis cell (LC) in the ventricle. Note the extension (arrows) emanating from the hyaline capsule and extending into the partial cellular capsule. Stained with PAS. X 350.
6. Light micrograph of a lymphocystis cell (LC) in plastic-embedded ventricle prepared for electron microscopy. The patches (arrow) present in the LC are large aggregations of virions. H, hemocytes; My, myocardium; CE, capsular extension. Stained with toluidine blue. X 1,170.
7. Electron micrograph of a section through one of the patches shown in Figure 6. Note the delicate radial-fibrillar region (arrows) that surrounds each virion (compare with Figure 11). The uniform spacing of closely-packed virions evidently is controlled by the radial-fibrillar region as concluded by Walker (1962) and Walker and Weissenberg (1965). X 50,500.



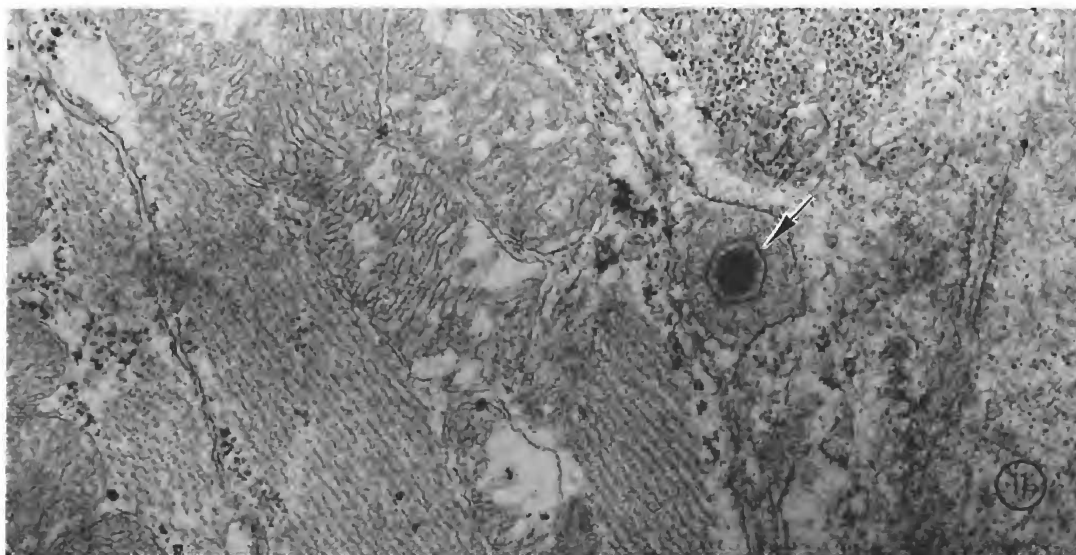
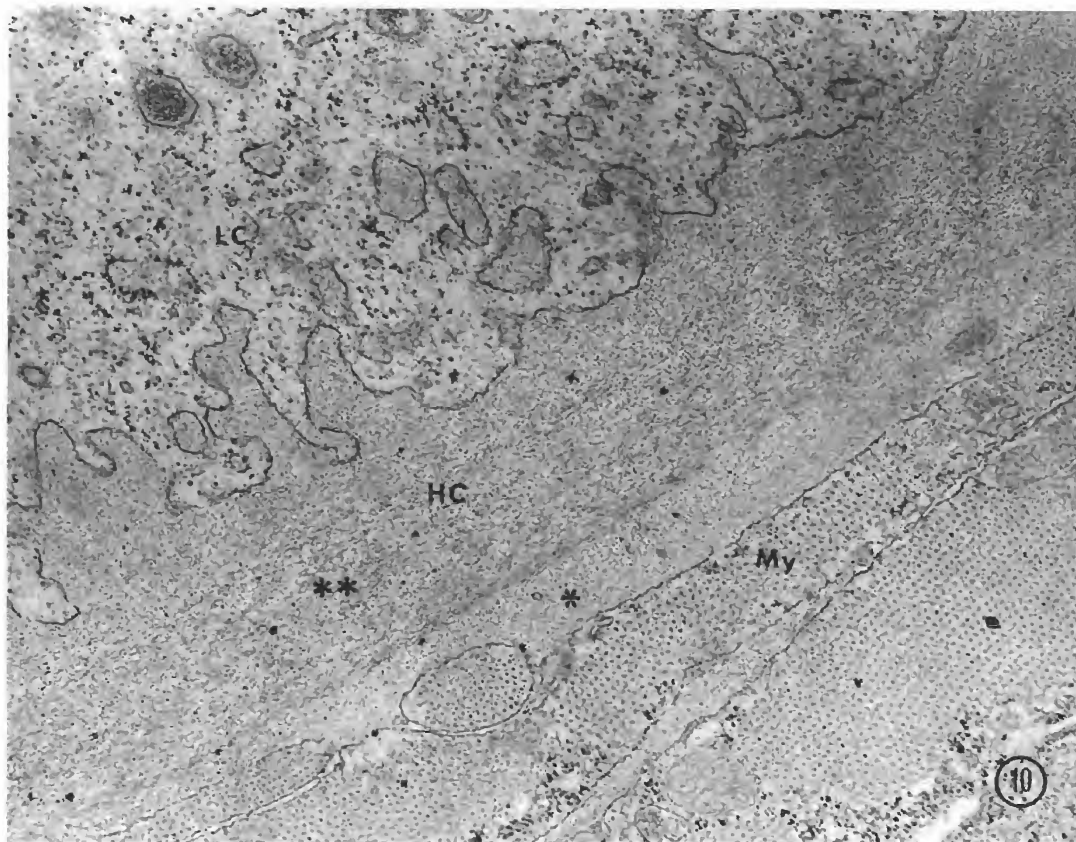
**PLATE 4****EXPLANATION OF FIGURES**

8. Section through the ventricle showing the hyaline capsule (HC) of a lymphocystis cell (LC) in contact with cells (EC) of the encapsulating envelope. M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; V, virus. X 12,500.
9. Electron micrograph of capsular extension (CE) which almost encircles a cell of the encapsulating envelope. Note fibrils (arrows) in the perinuclear region of the cell. HC, hyaline capsule; RER, rough endoplasmic reticulum. X 26,300.



**PLATE 5****EXPLANATION OF FIGURES**

10. Section showing the irregular periphery of a lymphocystis cell (LC), the hyaline capsule (HC) and cells of the ventricular myocardium (My) to which the capsule is apposed. Note that the capsule is divided into a thick inner (\*\*\*) and a thin outer (\*) layer by the orientation of its component fibrils. X 38,900.
11. Section through the ventricular myocardium showing a single virus particle (arrow) in the intercellular space. The particle is surrounded by a granular substance. X 44,000.

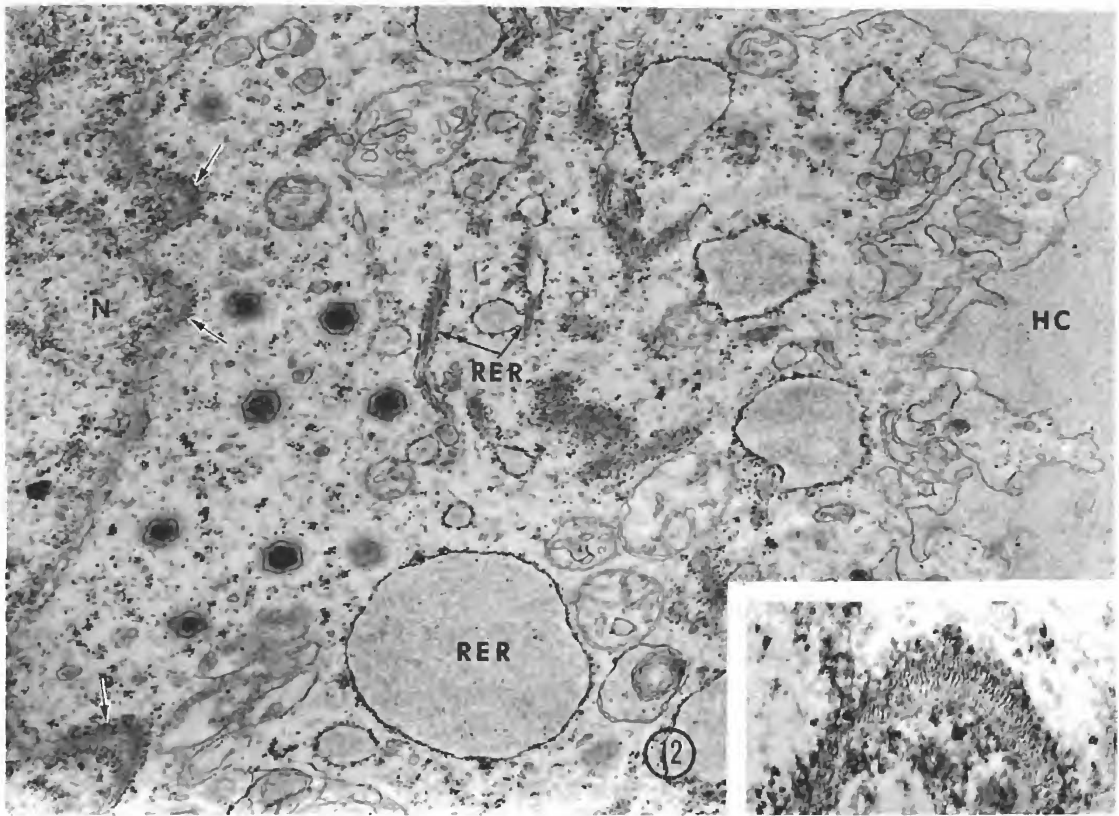


## PLATE 6

## EXPLANATION OF FIGURES

12. Electron micrograph of a lymphocystis cell. Some elements of the rough endoplasmic reticulum (RER) are markedly dilated and others contain cross-banded fibrils. Note fibrils present in the perinuclear cisterna (arrows). N, nucleus; HC, hyaline capsule. X 23,300. Inset: Higher power view of fibrils in the perinuclear cisterna. X 70,400.
13. Electron micrograph showing apparently active elements of the rough endoplasmic reticulum (RER). Note the fibril (arrow) extending into the granular substance of the dilated element. M, mitochondria. X 26,700.





**PLATE 7****EXPLANATION OF FIGURES**

14. Section through the periphery of a lymphocystis cell showing annulate lamellae (AL). Note continuity of AL with the RER (arrows). HC, hyaline capsule. X 35,200.
15. Higher power electron micrograph showing continuity of annulate lamellae with RER (arrow). X 80,500.
16. Annulate lamellae (AL) adjacent to cisternae of rough endoplasmic reticulum (RER) that contain cross-banded fibrils. X 80,400.

